

Attorney Docket No. 9237.23
Applicant Serial No: 10/089,595
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IN THE SPECIFICATION

Please amend the specification as follows.

On page 70, line 32 through page 71, line 20, please amend the paragraph as follows.

Molecular beacon technology to detect polymorphisms was according to published protocols (Tyagi *et al.*, 1998; Kostrikis *et al.*, 1998; Piatek *et al.*, 1998; Tyagi and Kramer, 1996) ~~and those found at www.molecular-beacons.org.~~ Time-to-event statistical issues and other pertinent analyses were performed according to published protocols (Dolan *et al.*, 1993; 1995). In large part, the methods utilizing molecular tools for HLA-typing use commercially available reagents/kits, and it is relatively easy to do large numbers of samples in a high-through-put fashion. The technique is based on PCR amplification with sequence-specific primers and subsequent hybridization with sequence-specific oligonucleotide probes (PCR-SSOP; (Bozon *et al.*, 1996)). In brief, using locus-specific primers, different regions of short arm of chromosome 6 (HLA loci) are PCR amplified in 100µl reactions. After confirming the fidelity of the PCR reaction, 5 µl of the amplicon is dot-blotted to a positively charged nylon membrane using a multi-channel pipettor. The membranes are air-dried, denatured, cross-linked, and then hybridized with alkaline phosphatase-labeled oligonucleotide probes (LifeCodes). Non-specific hybridization is removed by pre-washing the membranes with TMAC followed by treatment with Lumiphos 480 (Life Codes, Stamford, CT), and then exposed to x-ray film. Using a DOT scan computer program (Life Codes), the hybridizing signals are coded by the program and allele(s) assigned. Based on the hybridizing patterns, the computer program resolves homozygosity or heterozygosity. The hybridization is performed in two steps. In the first step, oligonucleotide probes that resolve the haplotypes at low resolution are used. The results obtained at this point are generally comparable to that reported previously by serological methods. For higher resolution of the alleles, another round of hybridization is performed using locus-specific oligonucleotides.

On page 99, lines 3-11, please amend the paragraph as follows.

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A second mechanism includes the possibility that differences in the secondary structures of the 5'-UTRs of the distinct CCR5 transcripts may influence translation efficiency. It is known that a Gibbs free energy of formation (ΔG) of less than -50 kcal/mol can impair the passage of the ribosomal 40S subunits as they scan from the cap site (Kozak, 1986). Algorithms developed by Zuker (Zuker, 1989 and <http://www.ibc.wustl.edu/~zucker/ma>) were used to analyze the 5'-UTRs of CCR5A and CCR5B for their tendency to undergo secondary structure. These algorithms predict that the ΔG of CCR5A and CCR5B are -69.5 kcal/mol and -48.7 kcal/mol, respectively, suggesting that relative to CCR5B, CCR5A has a higher propensity to form a very stable structure.

On page 152, lines 21-32, please amend the paragraph as follows.

Methods for molecular beacon-based genotyping assays (Tyagi *et al.*, 1998; Kostrikis *et al.*, 1998; <http://www.molecular-beacons.org/>) were used for genotyping CCR5 T627C and A676G. An example for real-time monitoring of PCR for genotyping of CCR5 627 (C/T) was developed. Real-time measurements of CCR5 amplicon synthesis from DNA samples that are homozygous C/C (red), homozygous T/T (green) or heterozygous C/T (blue) were observed. DNA samples were amplified and detected as either molecular beacons complementary to CCR5 627C labeled with fluorescein or to CCR5 627T labeled with tetrachlorofluorescein (TET). The molecular beacon assay method was as described (Tyagi *et al.*, 1998). PCR amplifications were performed in a 7700 Prism spectrofluorometric thermal cycler (Perkin-Elmer) for 45 cycles with the following conditions: 95C for 30 s, 55C (CCR5 627) or 50C (CCR5 676) annealing for 60 s, and 72C for 30 s. Fluorescence was measured during the 60 s annealing step in each thermal cycle.